Translation

PCT Application
PCT/JP2003/002394

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT-NU-006	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP2003/002394	International filing date (*day/month/year*) 28 February 2003 (28.02.2003)	Priority date (*day/month/year*) 04 March 2002 (04.03.2002)
International Patent Classification (IPC) or national classification and IPC C07K 14/80, 7/06, 7/08, 1/12, 1/16, A61K 38/17, A61P 3/00, 7/00, 9/00, 43/00		
Applicant NIHON UNIVERSITY SCHOOL JURIDICAL PERSON		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 07 May 2003 (07.05.2003)	Date of completion of this report 14 January 2004 (14.01.2004)
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP2003/002394

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-11, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the claims:

pages 1-3, as originally filed

pages _____, as amended (together with any statement under Article 19

pages _____, filed with the demand

pages 4-22, filed with the letter of 25 November 2003 (25.11.2003)

☐ the drawings:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1/5-5/5, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-22	YES
	Claims		NO
Inventive step (IS)	Claims	18-22	YES
	Claims	1-17	NO
Industrial applicability (IA)	Claims	1-22	YES
	Claims		NO

2. Citations and explanations

Document 1: Samya Othman *et al.*, "Resonance Raman investigation of lysine and N-acetyl-methionine complexes of ferric and ferrous microperoxidase", European Biophysics Journal, 1999, Vol. 28 (1), pp. 12-25; see entire document

Document 2: Jin-Shyan Wang *et al.*, "Temperature- and pH-dependent changes in the coordination sphere of the heme c group in the model peroxidase N^{α}-acetyl microperoxidase-8", The Journal of Biological Chemistry, 1992, Vol. 267 (22), pp. 15310-15318; see entire document

Document 3: Shyamalava Mazumdar *et al.*, "Stability and characterization of iron(III) and iron(II) heme peptides encapsulated in aqueous detergent micelles: ^{1}H NMR and UV-Vis spectroscopic studies", Inorganic Chemistry, 1991, Vol. 30 (4), pp. 700-705; see entire document and especially Fig. 1

Document 4: Seiji Yamada et al., "Characterization and Amino Acid Sequences of Cytochromes c_6 from Two Strains of the Green Alga *Chlorella vulgaris*", Bioscience, Biotechnology and

Biochemistry, 2000, Vol. 64(3), pp. 628-632, see entire document

Document 5: Richard E. Dickerson *et al.*, "Ferricytochrome c", The Journal of Biological Chemistry, 1971, Vol. 246 (5), pp. 1511-1535; entire document and especially Fig. 1 and appendix

Document 6: Seiji Yamada *et al.*, "Structure of cytochrome c_6 from the red alga *Porphyra yezoensis* at 1.57Å resolution", Acta Crystallographica Section D: Biological Crystallography, 2000, Vol. D56 (12), pp. 1577-1582; see entire document and especially Fig. 4

Document 7: "Issanka chisso (NO) no 'heme' tanpakushitsu ni yoru hosoku", Kagaku to Seibutsu, 1996, Vol. 34 (12), pp. 784-785; see entire document

Document 1 discloses a heme peptide in which heme c is coordinated with porphyrin and the peptide MP8, and also mentions that it is stabilized when the His of the Cys-Ala-Gln-Cys-His peptide derived from MP8 is bound to the heme c.

Document 2 discloses MP-8 as an octapeptide comprising Cys-Ala-Gln-Cys-His-Thr-Val-Glu, obtained by degrading cytochrome c, and mentions that via the His residue MP-8 forms one of the ligands of an iron complex comprising six ligands.

Document 3 discloses treatment of cytochrome c to obtain a heme octapeptide comprising Cys-Ala-Gln-Cys-His-Thr-Val-Glu, and treatment with chymotrypsin to give a heme undecapeptide comprising Val-Glu-Lys-Cys-Ala-Gln-Cys-His-Thr-Val-Glu.

Document 4 indicates that the heme c binding domain of cytochrome c has a -CXXCH- motif.

Document 5 discloses equine cytochrome c.

Document 6 discloses cytochrome c from the red alga *Porphyra yezoensis*.

Document 1 discloses an iron complex in which MP8 and porphyrin are ligands, and documents 1 and 2 disclose stabilization when MP8 is coordinated via the histidine residue thereof; therefore, a person skilled in the art could easily conceive of obtaining an iron complex having MP8 and porphyrin as ligands.

In addition, it was commonly known before the priority date of the present application that when treated with trypsin or chymotrypsin, cytochrome c gives a peptide which includes the sequence Cys-Ala-Gln-Cys-His, as disclosed in document 3, and that a sequence comprising -CXXCH- is a heme binding motif, as disclosed in document 4. Therefore, a person skilled in the art could easily treat cytochrome c with a restriction enzyme such as trypsin and use a method of purification such as chromatography to obtain a heme peptide comprising a peptide which includes a -CXXCH- motif such as Cys-Ala-Gln-Cys-His. Selection of suitable known and conventionally used methods for purification for this purification in order to raise the purity of the protein is within the ordinary competence of a person skilled in the art. Selection of cytochrome from the horse or from *Porphyria yezoensis*, as disclosed in documents 5-6, as the cytochrome c starting material is also within the ordinary competence of a person skilled in the art.

Therefore, claims 1-17 could be derived easily by a

person skilled in the art from documents 1-5.

Document 7 indicates that higher NO capturing capacity can be obtained by modification of the heme protein cytochrome c.

The inventions set forth in claims 18-22 are not disclosed in any of the documents cited in the international search report, and are novel. Document 7 indicates that higher NO capturing capacity can be obtained by modification of the heme protein cytochrome c, but does not mention that a heme peptide acts to capture NO, and this feature could not be deduced easily by a person skilled in the art.